

## Monitoring the Fermentation of cv. Kalamata Natural Black Olives Using Raman Spectroscopy

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### INTRODUCTION & AIM

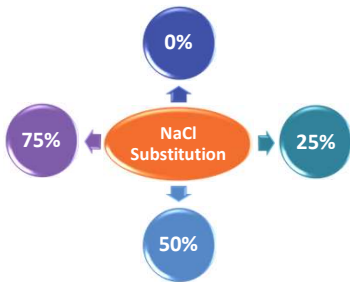
Fermented olives hold considerable economic importance for the Mediterranean countries [1]. Kalamata natural black olives are renowned for their appealing organoleptic properties and nutritive value [2]. The aim of this study was to monitor the efficacy of Raman spectroscopy as a rapid and non-invasive technique to monitor the fermentation of cv. Kalamata olives under diverse substitution levels of NaCl by KCl in the brine.

### METHOD

✓ Kalamata natural black olives were subjected to Greek-style processing according to the traditional anaerobic method in 7% brine.

✓ Partial substitutions of NaCl by KCl were performed.

✓ All fermentations were carried out in duplicate at 20–22°C for 145 days.

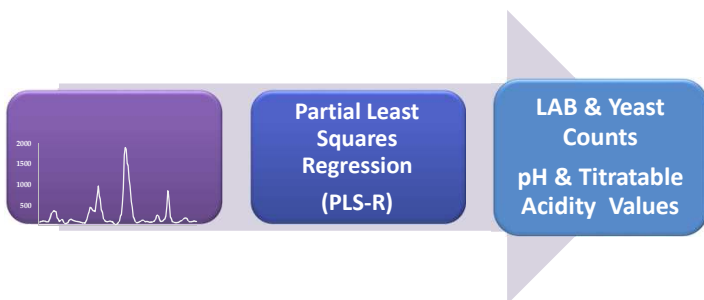


### Raman Spectra Acquisition



✓ Raman spectra were acquired twice a week from the surface of fermenting olives.

✓ Spectra were averaged and the area from 1800 to 1000  $\text{cm}^{-1}$  was included in the analysis.



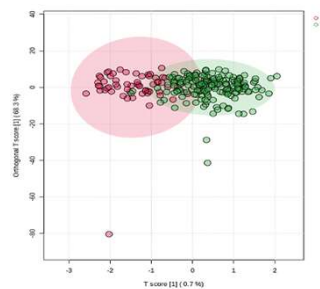
### RESULTS & DISCUSSION

The plot of scores (Figures 1 and 2), illustrate on the horizontal axis the class separation (i.e., NaCl vs. NaCl/KCl or beginning vs. end of fermentation, respectively), against the within class variation depicted in the vertical axis.

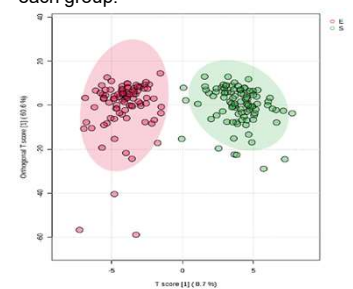
➤ The analysis reveals two distinct clusters for control fermentation against NaCl/KCl fermentations with some degree of overlapping (Figure 1).

➤ Distinct separation of classes was obtained along the horizontal axis between samples corresponding to the beginning and end of fermentation, with no overlapping at the 95% confidence interval level (Figure 2).

**Figure 1.** OPLS-DA Scores Plot Comparing Control (C) and NaCl substitution by KCl (S) in cv. Kalamata olive samples. Ellipses indicate the 95% confidence interval for each group.



**Figure 2.** OPLS-DA Scores Plot comparing cv. Kalamata olives from all substitution levels at the beginning (S) and end (E) of fermentation. Ellipses indicate the 95% confidence interval for each group.



**Table 1.** Performance Metrics of Calibration and Cross-Validation for the PLS-R Models. The statistical outputs include Latent Variables (LVs),  $R^2$  for Calibration ( $R^2_c$ ), Cross-Validation ( $R^2_{cv}$ ), and the Root Mean Squared Error (RMSE) of calibration and validation.

| Model   | LAB              | LVs | $R^2_c$ | $RMSE_c$ | $R^2_{cv}$ | $RMSE_{cv}$ |
|---------|------------------|-----|---------|----------|------------|-------------|
| LAB     | SNV <sup>1</sup> | 6   | 0.75    | 0.68     | 0.64       | 0.83        |
| Yeasts  | Raw data         | 5   | 0.51    | 0.52     | 0.44       | 0.58        |
| pH      | SNV <sup>1</sup> | 5   | 0.72    | 0.33     | 0.65       | 0.38        |
| Acidity | Raw data         | 6   | 0.73    | 0.089    | 0.56       | 0.11        |

<sup>1</sup> Standard Normal Variate

The best performance of PLS-R models is presented in Table 1 for each parameter measured. The results of validation are satisfactory but additional experiments are required to increase the robustness of models.

### CONCLUSION

Overall, the results obtained in this work provided promising perspective for the utilization of Raman spectroscopy as a rapid and non-invasive technique to monitor table olive fermentation. However, substantial model validation through further studies are required.

### FUTURE WORK / REFERENCES

- Kazou, M.; Tzamourani, A.; Panagou, E.Z.; Tsakalidou, E. Unraveling the Microbiota of Natural Black Cv. Kalamata Fermented Olives through 16S and ITS Metataxonomic Analysis. *Microorganisms* 2020, 8, 672.
- Rocha, J.; Borges, N.; Pinho, O. Table olives and health: a review. *J. Nutr. Sci.* 2020, 9, e57.